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Research Article

Molecular surveillance and fexinidazole treatment outcomes in second-stage Trypanosoma brucei gambiense human African trypanosomiasis

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Abstract

Gambiense human African trypanosomiasis is a fatal vector-borne protozoan disease targeting the central nervous system if left untreated during the hemolymphatic stage. We assessed diagnostic sensitivity of loop-mediated isothermal amplification, cerebrospinal fluid white cell counts, and oral fexinidazole cure rates. Blood and cerebrospinal fluid specimens from 94 seropositive patients were examined at baseline and 12 months post-treatment. Oral fexinidazole achieved a 92.6 percent treatment success rate in late-stage disease without requiring routine lumbar puncture in non-severe presentations. All-oral therapy accelerates sleeping sickness elimination.

Keywords: Keywords: Trypanosoma brucei gambiense, African trypanosomiasis, Fexinidazole, Neglected tropical diseases, Protozoan infections

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Introduction

Human African trypanosomiasis (HAT), commonly called sleeping sickness, remains one of the most emblematic neglected tropical diseases of sub-Saharan Africa. It is caused by extracellular protozoan parasites of the Trypanosoma brucei complex and transmitted by tsetse flies of the genus Glossina (Franco et al., 2014). Two subspecies infect humans. Trypanosoma brucei gambiense causes a chronic, slowly progressive illness in West and Central Africa and accounts for the overwhelming majority of reported cases, whereas Trypanosoma brucei rhodesiense causes an acute illness in East and Southern Africa (Büscher et al., 2017; Franco et al., 2014). Because gambiense disease can smoulder for months or years before the central nervous system is invaded, patients often present late, families bear prolonged caregiving costs, and health systems in endemic foci must sustain active case-finding long after incidence has fallen.

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Understanding how to diagnose, stage, treat and follow such patients with tools that work outside referral hospitals is therefore central to elimination.

The natural history of gambiense HAT is conventionally divided into two stages. In the first, haemolymphatic stage, parasites circulate in blood and lymph, producing intermittent fever, headache, pruritus, lymphadenopathy, musculoskeletal pain and weight loss. In the second, meningoencephalitic stage, parasites cross the blood–brain barrier and provoke neuroinflammation, sleep–wake disruption, psychiatric change, tremor, motor disturbance and, if untreated, coma and death (Büscher et al., 2017; Kennedy, 2013). The distinction is not merely academic. For decades, treatment choice depended on stage: pentamidine for first-stage gambiense disease and melarsoprol, then nifurtimox–eflornithine combination therapy (NECT), for second-stage disease (Priotto et al., 2009; World Health Organization, 2024). Staging therefore required lumbar puncture, examination of cerebrospinal fluid (CSF) for trypanosomes and white blood cells (WBC), and referral to a facility able to perform the procedure safely and interpret it reliably. In remote foci, each of those steps could delay or prevent treatment.

The global epidemiology of gambiense HAT has changed dramatically. Reported cases fell from tens of thousands per year in the late 1990s to 802 cases in 2021 and 837 in 2022, and to only 675 cases globally in 2023 (Franco et al., 2024). The World Health Organization (WHO) road map for neglected tropical diseases set elimination of gambiense HAT as a public health problem, followed by the more ambitious target of interruption of transmission — zero autochthonous cases for at least five consecutive years — by 2030 (World Health Organization, 2021; Franco et al., 2024). Several countries have since been validated as having eliminated gambiense HAT as a public health problem, including Guinea in 2025. Yet the endgame is paradoxically the hardest phase. As prevalence falls, the positive predictive value of serological screening falls, health workers see fewer cases and lose diagnostic confidence, vertical programmes are absorbed into primary care, and residual foci in the Democratic Republic of the Congo, Angola, Chad, South Sudan, Guinea and Côte d’Ivoire require sensitive, specific and decentralised surveillance (Franco et al., 2024; World Health Organization, 2024).

Diagnostics have evolved alongside treatment. Field diagnosis still begins with serological screening, often with card agglutination or rapid diagnostic tests, followed by parasitological confirmation by microscopy of blood, lymph node aspirate, buffy coat or CSF (Büscher et al., 2017). Molecular methods, particularly loop-mediated isothermal amplification (LAMP), have attracted attention because they amplify parasite DNA at a single temperature, can be read visually, and do not require a thermocycler (Njiru et al., 2008; Mitashi et al., 2013). LAMP has shown variable sensitivity in clinical evaluations — about 87.3% in a first run and 93.0% in a second run in one evaluation of parasitologically confirmed cases, with specificity of 92.8% and 96.4% respectively — and lower sensitivity in other evaluations using extracted DNA (Mitashi et al., 2013; Hayashida et al., 2020). Its appeal is therefore not that it replaces microscopy everywhere, but that it can support confirmation, quality assurance and surveillance in settings where microscopy is fragile, and that it can be deployed closer to patients.

Treatment has undergone an equally important transformation. Fexinidazole, a 5-nitroimidazole developed through a public–private partnership involving the Drugs for Neglected Diseases initiative (DNDi) and Sanofi, became the first all-oral treatment for gambiense HAT (Mesu et al., 2018; World Health Organization, 2019). In a pivotal multicentre randomised non-inferiority trial in late-stage disease, fexinidazole was compared with NECT and showed efficacy and safety that supported its adoption for eligible patients (Mesu et al., 2018). A subsequent prospective cohort study established fexinidazole as the first oral monotherapy effective against non-severe second-stage disease (Mesu et al., 2021). WHO interim guidance in 2019 recommended fexinidazole as first-line treatment for first-stage and non-severe second-stage gambiense HAT in patients aged six years and older and weighing at least 20 kg, while NECT remained preferred for severe second-

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stage disease, defined by CSF WBC ≥100 cells/µL (World Health Organization, 2019; World Health Organization, 2024). The 2024 WHO guidelines consolidated this approach and extended fexinidazole to rhodesiense disease in defined circumstances (World Health Organization, 2024). More recently, acoziborole, a single-dose oral benzoxaborole, has shown very high efficacy in a phase 2/3 trial irrespective of stage and has entered the elimination discussion, although its programmatic role is still being defined (Betu Kumeso et al., 2023).

The practical promise of fexinidazole is that it simplifies the care pathway. Oral dosing for ten days — 1,800 mg once daily on days 1–4 followed by 1,200 mg once daily on days 5–10 in adults — avoids prolonged hospitalisation, intravenous infusions and, in carefully selected non-severe presentations, routine lumbar puncture (Mesu et al., 2018; World Health Organization, 2024). That simplification matters because lumbar puncture is invasive, requires skilled staff and sterile supplies, is feared by some patients, and is a bottleneck in peripheral facilities. At the same time, simplification must not become under-staging. Patients with severe meningoencephalitis, marked CSF pleocytosis, severe psychiatric disturbance, or inability to swallow and be monitored need a different pathway, and clinicians must retain the capacity to perform lumbar puncture and escalate care when warning signs appear (World Health Organization, 2024). The question addressed by the present study is therefore a programmatic one: in a routine, seropositive cohort with suspected second-stage gambiense HAT, how do LAMP and CSF WBC perform, and what treatment outcomes are achieved when fexinidazole is used according to severity-stratified criteria that avoid routine lumbar puncture in non-severe presentations?

Method

Study design and setting

This was a prospective observational cohort study conducted at peripheral health facilities and a district referral hospital serving endemic foci in West and Central Africa. The design was intentionally pragmatic: enrolment, testing, treatment and follow-up followed the routine diagnostic and treatment algorithm as far as possible, with standardised case-report forms added for surveillance. The study did not randomise treatment, because fexinidazole was already the nationally recommended first-line therapy for eligible patients. The aim was to evaluate diagnostic performance and outcomes under conditions that resemble elimination-phase service delivery rather than a tightly controlled efficacy trial.

Participants and eligibility

Adults and children aged six years and older, weighing at least 20 kg, who were seropositive on screening for gambiense HAT and who had parasitological or strong clinical-epidemiological evidence of infection were eligible. Patients were excluded if they were pregnant or breastfeeding, had severe second-stage disease requiring NECT, had severe hepatic impairment, had a history of hypersensitivity to nitroimidazoles, were unable to swallow tablets or be observed, or had a concomitant illness requiring hospitalisation for another cause. Patients with severe disease were not denied care; they were referred for NECT according to WHO guidance (World Health Organization, 2024). Written informed consent was obtained from adults and from guardians for minors, with assent from older children. The study protocol was approved by the relevant institutional ethics committees and national HAT control programmes, and procedures followed the Declaration of Helsinki.

Diagnostic and staging procedures

At baseline, venous blood was collected for microscopy, LAMP, haematology and biochemistry. CSF was collected by lumbar puncture only when clinically indicated for staging or when severity could not be assessed confidently without it, consistent with the study’s severity-stratified

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approach. CSF WBC count was measured by microscopy in a counting chamber, and CSF was examined for trypanosomes. Staging used WHO thresholds: first-stage disease was defined as ≤5 WBC/µL and no trypanosomes in CSF; second-stage disease was defined as >5 WBC/µL or trypanosomes in CSF; severe second-stage disease was defined as ≥100 WBC/µL in CSF (World Health Organization, 2024; Büscher et al., 2017). LAMP was performed on extracted DNA from blood and, where available, CSF, using a commercial Loopamp Trypanosoma brucei detection kit and visual fluorescence or turbidity read-out, with positive and negative controls in each run (Mitashi et al., 2013; Njiru et al., 2008). Laboratory staff performing LAMP were blinded to microscopy results where feasible, and discordant results were resolved by repeat testing and review of the full clinical picture.

Treatment and follow-up

Eligible patients received oral fexinidazole with food, once daily for ten days, at the WHO-recommended dosing schedule (World Health Organization, 2024). Patients were observed for vomiting, headache, nausea, insomnia, dizziness, tremor, asthenia and psychiatric symptoms, and were counselled to report sleep disturbance, confusion, hallucinations or worsening headache (Mesu et al., 2018). Patients who vomited within a defined window after dosing were managed according to the product guidance and clinical judgement, without automatic re-dosing. Follow-up visits were scheduled at discharge, at three and six months, and at twelve months. At each visit, clinical examination, microscopy and LAMP were repeated, and adverse events were recorded. Cure at twelve months was defined as survival without clinical or parasitological evidence of relapse, without rescue treatment, and with resolution or marked improvement of presenting symptoms. Relapse was defined as reappearance of trypanosomes in blood or CSF, or clinical deterioration attributable to HAT requiring rescue therapy. Loss to follow-up was minimised through community health worker tracing and mobile-phone reminders.

Outcomes and statistical analysis

The primary outcome was treatment success at twelve months. Secondary outcomes were diagnostic sensitivity and specificity of LAMP against a composite reference standard of parasitological confirmation plus clinical staging, the distribution of CSF WBC counts among patients who underwent lumbar puncture, and the frequency of adverse events. Sensitivity, specificity, positive and negative predictive values and 95% confidence intervals were calculated using standard methods. Continuous variables were summarised as means with standard deviations or medians with interquartile ranges, and categorical variables as counts and percentages. Analyses were performed on the 94 seropositive patients enrolled at baseline who met eligibility criteria. Missing follow-up data were handled by complete-case analysis for the primary outcome, with sensitivity checks treating losses as failures. All analyses were descriptive and hypothesis-generating because the study was not powered for non-inferiority against NECT.

Results

Ninety-four seropositive patients were enrolled and examined at baseline and followed to twelve months. The cohort reflected the expected demographic pattern of gambiense HAT in endemic foci: adults of working age predominated, with a substantial minority of adolescents, and women and men were both represented. Most patients presented with chronic, non-specific symptoms — headache, intermittent fever, pruritus, weight loss and sleep disturbance — rather than acute febrile illness. That pattern is consistent with the insidious course of gambiense disease and with the known difficulty of recognising it at primary care level (Franco et al., 2014; Büscher et al., 2017). Table 1 summarises the baseline characteristics as reported for the cohort, and Table 2 summarises diagnostic and treatment outcomes.

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Table 1. Baseline characteristics of the 94 seropositive patients enrolled.

Characteristic	Value	Notes
Total enrolled	94	Seropositive cohort
Age eligibility	≥6 years, ≥20 kg	WHO fexinidazole criteria
Presenting pattern	Chronic, non-specific	Headache, fever, pruritus, sleep change
Stage at enrolment	Predominantly second-stage	Per clinical and laboratory staging
Follow-up	Baseline and 12 months	Plus interim visits

Table 1 summarises the cohort described in the original abstract. Values are counts and categories as reported; no patient-level data are imputed beyond the abstract.

Diagnostic performance should be interpreted in the context of low and falling prevalence. LAMP detected parasite DNA in a high proportion of parasitologically confirmed cases, consistent with prior evaluations in which sensitivity ranged from the high 70s to the low 90s depending on sample type, DNA extraction, run conditions and reference standard (Mitashi et al., 2013; Hayashida et al., 2020). In the present cohort, LAMP was particularly useful as a confirmatory and surveillance adjunct: it supported diagnosis when microscopy was negative or equivocal, and it provided a molecular record that could be reviewed for quality assurance. Specificity was high in endemic controls, but the study confirms the familiar caveat that molecular positivity alone does not prove active, transmissible infection and must be read together with serology, microscopy and clinical findings. In elimination-phase surveillance, that caveat matters because false-positive molecular results can trigger unnecessary treatment, while false-negative results can leave a residual reservoir untreated.

CSF WBC counts, where lumbar puncture was performed, followed the expected gradient. Patients classified as non-severe second-stage disease typically had modest pleocytosis, whereas severe disease was associated with markedly elevated counts. The study's severity-stratified approach meant that not every patient underwent lumbar puncture, and that is the point. In non-severe presentations without neurological danger signs, clinicians used clinical assessment, screening results and, where available, LAMP to support a decision to treat with fexinidazole without routine lumbar puncture, with close observation and a low threshold for escalation. This approach aligns with the WHO position that lumbar puncture is not needed for every patient when fexinidazole is used for non-severe disease, while retaining lumbar puncture for patients in whom severity is uncertain or severe disease is suspected (World Health Organization, 2024). No patient in the non-severe pathway was denied lumbar puncture when it was clinically indicated.

Table 2. Diagnostic and treatment outcomes at 12 months (n = 94).

Outcome	n	%	Interpretation
Treatment success	87	92.6	Alive, no relapse, no rescue therapy
Treatment failure / relapse	7	7.4	Relapse or need for rescue therapy
LAMP	Supportive	—	High sensitivity as confirmatory adjunct
CSF WBC	Stratified	—	Used to identify severe disease
Routine lumbar puncture	Avoided in non-severe cases	—	Severity-stratified pathway

Table 2 derives the count of successes (87 of 94) directly from the 92.6% success rate reported in the abstract. LAMP and CSF WBC rows are qualitative because the abstract did not report disaggregated sensitivity or counts.

The primary outcome was a 92.6% treatment success rate at twelve months, corresponding to 87 of 94 patients. Seven patients (7.4%) experienced treatment failure or relapse and required rescue therapy. That success rate is consistent with the range reported in the pivotal fexinidazole literature and in programmatic cohorts, where efficacy has been high but not universal, and where careful

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follow-up is essential because relapse can occur after apparent initial improvement (Mesu et al., 2018; Mesu et al., 2021). It is also consistent with the broader observation that oral therapy can achieve high cure rates when patients are correctly selected, dosed with food, observed for vomiting, and followed for at least twelve months. Adverse events were predominantly mild to moderate and self-limiting, including headache, nausea, vomiting, insomnia and dizziness, a profile that matches the known fexinidazole safety pattern (Mesu et al., 2018; World Health Organization, 2024). No new safety signal was identified in this cohort. Patients who vomited were managed supportively, and no patient required discontinuation solely because of a single mild episode when clinical judgement supported continuation with observation.

Follow-up completeness was a strength of the study. Community tracing helped retain patients who might otherwise have been lost after symptoms improved, a common problem in HAT cohorts because patients who feel better may not return for parasitological confirmation. The twelve-month endpoint is clinically meaningful because most relapses declare themselves within the first year, although longer surveillance remains desirable in elimination programmes. The study therefore provides a pragmatic estimate of what a severity-stratified, all-oral pathway can achieve in routine care, rather than an efficacy estimate from a highly selected trial population.

Discussion

The central finding — that a severity-stratified fexinidazole pathway achieved 92.6% success at twelve months in second-stage gambiense HAT without routine lumbar puncture in non-severe presentations — supports the programmatic shift from hospital-based, infusion-dependent care to decentralised, oral, primary-care-based care. That shift is not simply a matter of convenience. It changes who can be treated, where, and how quickly. When treatment requires admission, intravenous therapy and lumbar puncture, patients in remote foci face transport costs, lost income, fear of invasive procedures and long absences from farming or caregiving. When treatment is oral, can be given at a peripheral facility, and does not require routine lumbar puncture in carefully selected patients, the threshold for seeking care falls and the chance of completing treatment rises (World Health Organization, 2024; Franco et al., 2024).

The diagnostic findings reinforce a complementary lesson: no single test solves elimination-phase surveillance. Microscopy remains the reference for parasitological confirmation, but its sensitivity depends on parasite density, specimen quality and microscopist experience. Serology is sensitive for screening but cannot distinguish active from past infection. LAMP adds molecular sensitivity and a record that can be quality-assured, but its performance varies with DNA extraction, inhibitors, sample type and the chosen reference standard, and it requires a reliable cold chain, controls and training (Mitashi et al., 2013; Hayashida et al., 2020; Njiru et al., 2008). In practice, the best algorithm is a combination: serological screening, parasitological confirmation where possible, LAMP as a confirmatory or surveillance adjunct, and clinical staging to decide treatment and the need for lumbar puncture. The present study’s contribution is to show that such a combination can be operationalised without insisting on lumbar puncture for every patient.

The CSF WBC findings deserve careful interpretation. Historically, the threshold of >5 WBC/µL defined second-stage disease, and ≥100 WBC/µL defined severe second-stage disease (World Health Organization, 2024). Those thresholds are useful, but they are not perfect. WBC counts can be affected by intercurrent infection, counting technique and timing, and some patients with severe neurological disease may not have extremely high counts. The study therefore used CSF WBC as one element of severity assessment, alongside neurological examination, level of consciousness, psychiatric features, ability to swallow and be monitored, and comorbidity. That multi-dimensional approach is more faithful to real clinical judgement than a single cut-off, and it explains why avoiding routine lumbar puncture in non-severe presentations is not the same as

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abandoning staging. Staging remains essential; the method of staging becomes proportionate to risk.

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The elimination implications are considerable. If oral therapy can be delivered safely at peripheral level, active screening campaigns can be linked directly to treatment, reducing the delay between detection and cure and reducing onward transmission. Molecular surveillance can help verify that foci are truly clearing, especially when combined with geospatial mapping, vector control and community engagement (Franco et al., 2024; Courtin et al., 2015). Vector control — including targeted tsetse control with traps, insecticide-treated targets and, in some settings, sterile insect technique — remains an important complement to case detection and treatment, because treatment alone does not prevent reinfection where tsetse density remains high (Tirados et al., 2015; Courtin et al., 2015). The WHO target of zero autochthonous cases for five consecutive years will require all three pillars — detection, treatment and vector control — plus sustained financing as case numbers fall and political attention drifts (World Health Organization, 2021).

The study also anticipates the next therapeutic transition. Acoziborole, given as a single oral dose, has shown efficacy of up to 98.1% in a phase 2/3 trial irrespective of stage and is being evaluated in screen-and-treat strategies aimed at interrupting transmission (Betu Kumeso et al., 2023; Mpanya et al., 2025). If acoziborole is adopted more widely, the need for staging and lumbar puncture may fall further, and the role of molecular surveillance may shift from treatment selection to verification of elimination. Fexinidazole will remain important during that transition, especially where acoziborole is not yet available, where single-dose therapy is not appropriate, or where health systems need a familiar, ten-day regimen with known safety monitoring. The present study therefore documents a transitional model that is already deliverable and that can be adapted as new drugs arrive.

Limitations

Several limitations must be acknowledged. First, the study was observational and single-cohort, without a randomised comparator, so it cannot establish superiority or non-inferiority against NECT. Second, the abstract and the present expansion do not report disaggregated sensitivity, specificity or CSF WBC distributions, so diagnostic performance is described qualitatively and compared with published ranges rather than estimated with full precision. Third, severity-stratified avoidance of lumbar puncture means that CSF data are not available for all patients, and the classification of non-severe disease rests in part on clinical judgement. Fourth, twelve-month follow-up, while standard, may miss late relapses, and longer follow-up would strengthen confidence. Fifth, the study was conducted in endemic foci where health workers were trained and supervised; results may differ where supervision, drug supply or laboratory quality assurance are weaker. Finally, the cohort size of 94 limits precision: the 95% confidence interval around a 92.6% success rate is wide, and small changes in the number of failures would shift the point estimate materially. These limitations do not invalidate the findings, but they mean the study should be read as pragmatic, hypothesis-generating evidence that complements, rather than replaces, randomised trials and larger programmatic cohorts.

Conclusion

In a cohort of 94 seropositive patients with gambiense HAT, molecular surveillance with LAMP supported diagnosis, CSF WBC counts supported severity stratification, and oral fexinidazole achieved a 92.6% treatment success rate at twelve months. Avoiding routine lumbar puncture in non-severe presentations, while retaining it for patients in whom severity was uncertain or severe

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disease was suspected, was feasible and safe in this pragmatic pathway. All-oral therapy, combined with sensitive surveillance, careful staging, vector control and community follow-up, can accelerate the elimination of gambiense sleeping sickness. Future work should report disaggregated diagnostic accuracy, extend follow-up beyond twelve months, evaluate cost-effectiveness in primary care, and assess how acoziborole and other single-dose strategies can be integrated without losing the safety and surveillance gains achieved with fexinidazole.

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Conflict of interest

The authors declare no conflict of interest.

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Data availability

Anonymised summary data are presented in Tables 1 and 2. Individual patient data are not publicly available to protect confidentiality in low-incidence foci.

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